At the moment, it is necessary to provide a stream of nanocrystals to obtain thousands of single diffraction patterns which are merged and combined to datasets. With improved refinement methods like DEN (dynamic elastic networks) the obtained structures are now subject for not phase biased refinement up to 7 Å resolution [4].

In photosystem II the Mn_4O_5Ca cluster from the water oxidizing complex (WOC) will be investigated in respect of structural transitions between the S-States during the oxidizing of water to evolve molecular oxygen.

References

[1] H. Chapman, et al., Nature 470 (7332) (Feb 3 2011) 73–77.
[2] S. Boutet, et al., Science (May 31 2012).
[3] A. Aquila, et al., Opt Express. 20 (3) (Jan 30 2012) 2706–2716.
[4] A. Brunger, et al., Structure 20 (2012) 957–966.

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2P3

Hydrogen bonding and spin density distribution in the Q_B semiquinone of bacterial reaction centers and comparison with the Q_A site

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In the photosynthetic reaction center from *Rhodobacter sphaeroides*, the primary (Q_A) and secondary (Q_B) electron acceptors are both ubiquinone-50 (Q-10), but with very different properties and functions. Q_B is always seen to occupy the proximal location when the RC was frozen under illumination — indicating that it is this conformation which traps the semiquinone (SQ_B). Structures with Q_B in the proximal position show HN_δ of His-L190 as a potential H-bond donor to the carbonyl oxygen O_4, and backbone NH groups from Ile-L224 and/or Gly-L225 plus the OH group of Ser-L223 as potential H-bond donors to the O_1 carbonyl oxygen. The presence of a H-bond donation from the OH group of Ser-L223 is debatable with reports appearing for and against the presence of such a bond to the O_1 of the quinone or the SQ_B. To study interactions of the SQ_B with a protein environment that imparts functional differences, we have applied X-band 1D and 2D ESEEM. ^{14}N and ^{15}N 2D ESEEM spectra clearly show two nitrogens interacting with the SQ_B, each carrying transferred unpaired spin density. Quadrupole coupling constants indicate them to be a protonated nitrogen of a histidine residue and the amide nitrogen of a peptide group. Lines from exchangeable protons with three different anisotropic couplings were found in ^1H 2D ESEEM spectra. We also found negligible spectroscopic differences between the mutant with Ser-L223 changed to Ala and the wild type protein (WT), indicating only minor perturbations in the SQ_B spin density for the mutant. Qualitatively this suggests that a strong H-bond does not exist in the WT between the Ser-L223 OH group and the SQ_B O_1 atom in the Q_AQ_B state. We used QM/MM calculations on a Q_B site model to assign the ^1H and ^{14}N tensors to specific H-bond interactions with SQ_B, and we compared this with the SQ_A site. We showed quantitatively that a WT model in which the Ser-L223 OH group is rotated to prevent H-bond formation with the O1 atom of the SQ_B predicts negligible change for the mutant. This, together with the better agreement between key QM/MM calculated and experimental ^1H, ^{14}N, ^{13}C and ^{17}O hyperfine couplings for the non-hydrogen bonded model, leads us to conclude that no H-bond is formed between the Ser-L223 OH group and the SQ_B O_1 atom after the first flash. The calculations also reproduce a difference in the asymmetry of spin density distribution between SQ of the Q_A and Q_B sites, in agreement with data about ^{17}O and ^{13}C couplings of carbonyl groups.

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